

RIABLE REGION FRAGMENT ANTI-MOUSE IMMUNOGLOBULIN RSV19

SPECI  
E2  
VARIA 1 FIC FOR RESPIRATORY SYNCYTIAL VIRUS) /CN  
ANTI-IDIOTYPIC MONOCLONAL ANTIBODY B12 (OX HEAVY CHAIN

SPECIFIC  
BLE REGION FRAGMENT ANTI-MOUSE IMMUNOGLOBULIN RSV19

E3 0 --> FOR RESPIRATORY SYNCYTIAL VIRUS) /CN  
ANTI-IGE/CN

E4 1 ANTI-IL2R.ALPHA. MONOCLONAL ANTIBODY HEAVY CHAIN (HUMAN

E5 1 E 179 PRECURSOR REDUCED) /CN  
ANTI-IL2R.ALPHA. MONOCLONAL ANTIBODY HEAVY CHAIN (HUMAN

CLON  
CLON  
E 179 REDUCED) /CN

=> e "anti-igg"/cn 5

E1 1 ANTI-IDIOTYPIC MONOCLONAL ANTIBODY B12 (OX .LAMBDA.-CHAIN  
VA

RIABLE REGION FRAGMENT ANTI-MOUSE IMMUNOGLOBULIN RSV19

SPECI  
E2  
VARIA 1 FIC FOR RESPIRATORY SYNCYTIAL VIRUS) /CN  
ANTI-IDIOTYPIC MONOCLONAL ANTIBODY B12 (OX HEAVY CHAIN

SPECIFIC  
BLE REGION FRAGMENT ANTI-MOUSE IMMUNOGLOBULIN RSV19

E3 0 --> FOR RESPIRATORY SYNCYTIAL VIRUS) /CN  
ANTI-IGG/CN

E4 1 ANTI-IL2R.ALPHA. MONOCLONAL ANTIBODY HEAVY CHAIN (HUMAN

E5 1 E 179 PRECURSOR REDUCED) /CN  
ANTI-IL2R.ALPHA. MONOCLONAL ANTIBODY HEAVY CHAIN (HUMAN

CLON  
CLON  
E 179 REDUCED) /CN

=> e "anti-her-2"/cn 5

E1 1 ANTI-HEPTANAL ALDOXIME/CN  
E2 1 ANTI-HEPTANAL OXIME/CN  
E3 0 --> ANTI-HER-2/CN  
E4 1 ANTI-HIV-1 INTEGRASE SINGLE-CHAIN ANTIBODY (HUMAN) /CN  
E5 1 ANTI-HLA-DR ANTIGEN IG (MOUSE CLONE PMEK1HC .KAPPA.-CHAIN  
VK

III REGION FRAGMENT) /CN

=> e "anti-cd11a"/cn 5

E1 2 VARIABLE ANTI-CARCINOEMBRYONIC ANTIGEN ANTIBODY HEAVY CHAIN  
REGION (MOUSE) /CN

E2 1 VARIABLE ANTI-CARCINOEMBRYONIC ANTIGEN ANTIBODY LIGHT CHAIN  
REGION (MOUSE) /CN

E3 0 --> REGIO ANTI-CD11A/CN  
E4 1 REGIO ANTI-CD20 MONOCLONAL ANTIBODY 2B8 HEAVY CHAIN VARIABLE  
N (MOUSE PRECURSOR) /CN

E5 1 REGIO ANTI-CD20 MONOCLONAL ANTIBODY 2B8 LIGHT CHAIN VARIABLE  
N (MOUSE PRECURSOR) /CN

=> e "anti-cd18"/cn 5

E1 2 VARIABLE ANTI-CARCINOEMBRYONIC ANTIGEN ANTIBODY HEAVY CHAIN

REGION (MOUSE) /CN  
E2 1 ANTI-CARCINOEMBRYONIC ANTIGEN ANTIBODY LIGHT CHAIN  
VARIABLE  
REGION  
E3 0 --> ANTI-CD18/CN  
E4 1 ANTI-CD20 MONOCLONAL ANTIBODY 2B8 HEAVY CHAIN VARIABLE  
REGIO  
E5 1 ANTI-CD20 MONOCLONAL ANTIBODY 2B8 LIGHT CHAIN VARIABLE  
REGIO  
N (MOUSE PRECURSOR) /CN

=> e "anti-cd20"/cn 5  
E1 2 ANTI-CARCINOEMBRYONIC ANTIGEN ANTIBODY HEAVY CHAIN  
VARIABLE  
E2 1 ANTI-CARCINOEMBRYONIC ANTIGEN ANTIBODY LIGHT CHAIN  
VARIABLE  
REGION  
E3 0 --> ANTI-CD20/CN  
E4 1 ANTI-CD20 MONOCLONAL ANTIBODY 2B8 HEAVY CHAIN VARIABLE  
REGIO  
E5 1 ANTI-CD20 MONOCLONAL ANTIBODY 2B8 LIGHT CHAIN VARIABLE  
REGIO  
N (MOUSE PRECURSOR) /CN

=> s anti-cd20 ?/cn  
L1 2 ANTI-CD20 ?/CN

=> e "anti-vegf"/cn 5  
E1 1 ANTI-TRICYCLO(4.2.0.02,5)OCTADIENE/CN  
E2 1 ANTI-UV P/CN  
E3 0 --> ANTI-VEGF/CN  
E4 1 ANTIACID/CN  
E5 1 ANTIADIPOSITUM X 112/CN

=> e bovine serum albumin/cn 5  
E1 1 BOVINE RIBONUCLEASE A S-PROTEIN/CN  
E2 1 BOVINE SEMINAL RNASE (CATTLE ISOENZYME SUBUNIT PRECURSOR  
RED  
UCED) /CN  
E3 0 --> BOVINE SERUM ALBUMIN/CN  
E4 1 BOVINE SERUM ALBUMIN (330-337) /CN  
E5 1 BOVINE SERUM ALBUMIN (503-512) /CN

=> s bovine serum albumin ?/cn  
L2 3 BOVINE SERUM ALBUMIN ?/CN

=> s sodium chloride/cn  
L3 1 SODIUM CHLORIDE/CN

=> e serum albumin/cn 5  
E1 1 SERUKO/CN  
E2 1 SERUM .GAMMA.-GLOBULINS/CN  
E3 1 --> SERUM ALBUMIN/CN  
E4 1 SERUM ALBUMIN (115-GLUTAMINE) (HUMAN) /CN  
E5 1 SERUM ALBUMIN (115-HISTIDINE) (HUMAN) /CN

=> s e3  
L4 1 "SERUM ALBUMIN"/CN

=> fil medi,capplus,biosis,embase,wplids		
COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	0.00	-18.21

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=> s polypeptide monomer and (dimer or multimer) and (cation exchange or anion exchange) (w) chromatograph?

L5	0 FILE MEDLINE
L6	0 FILE CAPPLUS
L7	0 FILE BIOSIS
L8	0 FILE EMBASE
L9	0 FILE WPIDS

TOTAL FOR ALL FILES

L10	0 POLYPEPTIDE MONOMER AND (DIMER OR MULTIMER) AND (CATION EXCHANGE
-----	--

OR ANION EXCHANGE) (W) CHROMATOGRAPH?

=> s (polypeptide or antibody or l4 or l2 or serum albumin or anti ige or anti igg or "anti-her-2" or anti cd11a or anti cd18 or anti cd20 or anti vegf or l1) and ((ion or anion or cation) (w)exchange(w)resin)

L11	243 FILE MEDLINE
L12	252 FILE CAPPLUS
L13	113 FILE BIOSIS
L14	105 FILE EMBASE
L15	168 FILE WPIDS

TOTAL FOR ALL FILES

L16	881 (POLYPEPTIDE OR ANTIBODY OR L4 OR L2 OR SERUM ALBUMIN OR ANTI IGE OR ANTI IGG OR "ANTI-HER-2" OR ANTI CD11A OR ANTI CD18 OR ANTI CD20 OR ANTI VEGF OR L1) AND ((ION OR ANION OR CATION) (W) EXCHANGE(W) RESIN)
-----	--

=> s l16 and (sodium salt or l3 or sodium chloride or elution salt)

L17	6 FILE MEDLINE
L18	5 FILE CAPPLUS
L19	4 FILE BIOSIS
L20	2 FILE EMBASE
L21	3 FILE WPIDS

TOTAL FOR ALL FILES

L22 20 L16 AND (SODIUM SALT OR L3 OR SODIUM CHLORIDE OR ELUTION SALT)

=> dup rem 122

PROCESSING COMPLETED FOR L22

L23 13 DUP REM L22 (7 DUPLICATES REMOVED)

=> d cbib abs 1-13;s 116 and separat?

L23 ANSWER 1 OF 13 BIOSIS COPYRIGHT 1999 BIOSIS

1999:345852 Document No.: PREV199900345852. In vitro human immune reactivity of fast protein liquid chromatography fractionated Paracoccidioides brasiliensis soluble antigens. Diniz, Susana N.; Cisalpino, Patricia S.; Koury, Matilde C.; Andrade, Glauzia M. Q.; Nogueira, Maria G. S.; Goes, Alfredo M. (1). (1) Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, 30 161-970 Brazil. Microbes and Infection, (April, 1999) Vol. 1, No. 5, pp. 353-360. ISSN: 1286-4579. Language: English. Summary Language: English.

AB Soluble antigens of Paracoccidioides brasiliensis yeast cells (PbAg) were fractionated in a fast protein liquid chromatography (FPLC) system, using Q-Sepharose anion-exchange resin, in order to characterize antigenic fractions that could elicit cell reactivity and antibody recognition in human paracoccidioidomycosis (PCM). PbAg fractions were eluted by 20 mM Tris-HCl solution (pH 9.6) with an increasing gradient up to 1 M NaCl. The FPLC system was able to resolve 7 fractions, enumerated from 0 to VI, according to the elution on the NaCl gradient. The analysis of each fraction on SDS-PAGE showed that fractions 0 to V were constituted by multiple protein bands with molecular mass ranging from 18 to 114 kDa. Large amounts of nucleic acids were evidenced in fraction VI, as revealed by agarose gel stained with ethidium bromide. Sera from PCM patients presenting different clinical forms contained antibodies that recognized antigens in all fractions with the exception of fraction VI as detected by ELISA. Further studies were designed to investigate the capacity of these fractions to induce cell proliferation. It was demonstrated that fractions III and V (200 and 450 mM NaCl, respectively) stimulated a significant proliferative response of peripheral blood mononuclear cells, while fraction 0 induced the lowest proliferative response among patients with PCM, in either acute, acute treated, or chronic forms.

L23 ANSWER 2 OF 13 CAPLUS COPYRIGHT 1999 ACS

1997:453931 Document No. 127:68006 Separation of molecules from dilute solutions using composite chromatography media having high dynamic sorptive capacity at high flow rates.. Coffman, Jonathan L.; Girot, Pierre; Boschetti, Egisto (Biosepra, Inc., USA). PCT Int. Appl. WO 9718024 A1 19970522, 50 pp. DESIGNATED STATES: W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US17345 19961030. PRIORITY: US 1995-556313 19951113.

AB The chromatog. media comprise porous mineral oxide, polymeric, or polymer-coated mineral oxide supports which show a reversible high sorptive capacity and high intraparticle mass transfer rates. The modified porous media comprise porous inorg. (e.g., silica), polymer-coated inorg. (e.g., polystyrene-coated silica), or polymeric (e.g., polystyrene) sorbents that are passivated with a polymeric lattice to prevent non-specific adsorption of or interaction with biomols. These supports may be passivated by use of a passivation mixt. comprising a main

monomer, a passivating monomer, and a crosslinking agent, which upon polymn. results in substantial elimination of the undesirable non-specific

interaction with biomols. In an example, porous silica particles (40-100 .mu.m) were immersed in a monomer soln. of acrylamidomethylpropane sulfonic acid sodium salt, N,N'-methylene-bis-acrylamide, diethylaminoethylmethacrylamide, and N,N,N',N'-tetramethylethylenediamine, which was polymd. at 60-70.degree.C with ammonium persulfate. The resin was sepd. and dried, and the resulting cation exchange resin had a sorption capacity for insulin (in 70% EtOH) of 80 mg/mL, and exclusion limit of 30 Kd.

L23 ANSWER 3 OF 13 CAPLUS COPYRIGHT 1999 ACS

1992:527799 Document No. 117:127799 Separation of proteins and dyes with ion

exchangers. Johnson, Richard Alan; Quirk, Alan Victor; Woodrow, John Rodney (Delta Biotechnology Ltd., UK). PCT Int. Appl. WO 9204367 A1 19920319, 22 pp. DESIGNATED STATES: W: AU, CA, GB, JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2.

APPLICATION: WO 1991-GB1556 19910912. PRIORITY: GB 1990-19919 19900912.

AB A process for removing some or all of a protein-binding compd. from an aq.

liq. contg. the protein-binding compd. and a protein to which it can bind or is bound, comprises (1) exposing the liq. to a disrupting material (e.g. salt and a fatty acid) to disrupt the binding of the protein to the protein-binding material; (2) exposing the liq. to an ion exchange resin to bind the protein-binding material to the resin; and (3) sepg. the resin from the liq. The above process may

be

used in a process for prep. a protein by fermn. in which the fermn. medium contg. the protein is contacted with an immobilized protein-binding

compd.; the small proportion of protein-binding compd. eluting with the protein is removed using the ion exchange resin. Cibacron Blue 3-GA dye was sepd. from human serum albumin by disruption with buffer contg. 2 M NaCl and 1 M Na octanoate followed by chromatog. on Dowex-1 resin.

L23 ANSWER 4 OF 13 MEDLINE

92118879 Document Number: 92118879. Mechanism of the conformational transition of melittin. Goto Y; Hagihara Y. (Department of Biology, Faculty of Science, Osaka University, Japan.) BIOCHEMISTRY, (1992 Jan 28) 31 (3) 732-8. Journal code: A0G. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB It is known that, while melittin at micromolar concentrations is unfolded under conditions of low ionic strength at neutral pH, it adopts a tetrameric alpha-helical structure under conditions of high ionic strength, at alkaline pH, or at high peptide concentrations. To understand

the mechanism of the conformational transition of melittin, we examined in

detail the conformation of melittin under various conditions by far-UV circular dichroism at 20 degrees C. We found that the helical conformation

is also stabilized by strong acids such as perchloric acid. The effects of

various acids varied largely and were similar to those of the corresponding salts, indicating that the anions are responsible for the salt- or acid-induced transitions. The order of effectiveness of various monovalent anions was consistent with the electroselectivity series of anions toward anion-exchange resins,

indicating that the anion binding is responsible for the salt- or acid-induced transitions. From the NaCl-, HCl-, and alkaline pH-induced conformational transitions, we constructed a phase diagram of the anion- and pH-dependent conformational transition. The phase diagram was similar in shape to that of acid-denatured apomyoglobin [Goto, Y., & Fink, A.L. (1990) J. Mol. Biol. 214, 803-805] or that of the amphiphilic Lys, Leu model polypeptide [Goto, Y., & Aimoto, S. (1991) J. Mol. Biol. 218, 387-396], suggesting a common mechanism of the conformational transition. The anion-, pH-, and peptide concentration-dependent conformational transition of melittin was explained on the basis of an equation in which the conformational transition is linked to proton and anion binding to the titratable groups.

L23 ANSWER 5 OF 13 MEDLINE DUPLICATE 1  
92218581 Document Number: 92218581. Rapid, two-step purification process for

the preparation of pyrogen-free murine immunoglobulin G1 monoclonal antibodies. Neidhardt E A; Luther M A; Recny M A. (PROCEPT, Inc., Cambridge, MA 02139.. )JOURNAL OF CHROMATOGRAPHY, (1992 Jan 31) 590 (2) 255-61. Journal code: HQF. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB A cost-efficient process was specifically designed for the preparation of gram amounts of highly pure murine immunoglobulin (Ig) G1 monoclonal antibodies (mAbs). This rapid, simple and scalable purification process employs a unique binding and elution protocol for IgG1 mAbs on a silica-based, mixed-mode ion-exchange resin followed by conventional anion-exchange chromatography. mAbs are bound to BakerBond ABx medium at pH 5.6 directly from serum-supplemented hybridoma culture supernatants. Contaminating proteins and nucleic acids are removed by an intermediate wash at pH 6.5, followed by the specific elution of IgG1 mAbs with 100 mM Tris-HCl (pH 8.5). The mAb eluate is then loaded directly on to QAE-Sepharose Fast Flow medium and eluted with 10 mM sodium phosphate buffer (pH 7.4), containing 150 mM sodium chloride. The resulting IgG1 mAbs are greater than 98% pure, free from measurable endotoxin, formulated in a physiological buffer and suitable for in vivo applications.

L23 ANSWER 6 OF 13 MEDLINE DUPLICATE 2  
93044526 Document Number: 93044526. Purification of recombinant human secretory phospholipase A2 (group II) produced in long-term immobilized cell culture. Levin W; Daniel R F; Stoner C R; Stoller T J; Wardwell-Swanson J A; Angelillo Y M; Familletti P C; Crowl R M. (Department of Protein Biochemistry, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.. )PROTEIN EXPRESSION AND PURIFICATION, (1992 Feb) 3 (1) 27-35. Journal code: BJV. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Recombinant human secretory phospholipase A2 (Group II) was expressed in long-term culture of immobilized Chinese hamster ovary cells utilizing a continuous-perfusion airlift bioreactor. The bioreactor was continuously perfused with cell-culture medium supplemented with 5% fetal calf serum at an average flow rate of 5 liters/day for 30 days. Recombinant phospholipase A2, at concentrations ranging from 100 to 500 micrograms/liter, was purified to apparent homogeneity by an efficient two-step procedure involving a silica-based cation-exchange resin and hydrophobic interaction chromatography (greater than 65% recovery of phospholipase A2). The purified recombinant protein has an apparent molecular weight of 16 kDa, identical to that of purified human placental or synovial fluid

phospholipase A2, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Application of the purified protein onto several different gel filtration columns resulted in elution of the protein at molecular weights corresponding to 3.1-4.7 kDa, suggesting an interaction of the protein with the column resins. However, analytical ultracentrifugation experiments revealed that the protein behaves as a monomer (13.8-14.2 kDa) over a protein concentration range of approximately 10 micrograms/ml to 5 mg/ml. With autoclaved Escherichia coli membranes as substrate, the recombinant protein has catalytic properties (pH optimum, effects of bovine serum albumin, sodium chloride concentration, and requirement for calcium) similar to those of the protein purified from human placenta.

L23 ANSWER 7 OF 13 CAPLUS COPYRIGHT 1999 ACS

1991:601432 Document No. 115:201432 Protein adsorption at solid-liquid interfaces: part IV - effects of different solid-liquid systems and various neutral salts. Hajra, Susmita; Chatteraj, D. K. (Dep. Food Technol. Biochem. Eng., Jadavpur Univ., Calcutta, 700032, India). Indian J. Biochem. Biophys., 28(4), 267-79 (English) 1991. CODEN: IJBBBQ.

ISSN:

0301-1208.

AB Adsorption isotherms of bovine serum albumin (BSA) at solid-water interfaces have been studied as a function of protein concn., ionic strength of the medium, pH, and temp. using silica, barium sulfate, carbon, alumina, chromium, ion-exchange resins, and Sephadex as solid interfaces. In most cases, isotherms for adsorption of BSA attained the state of adsorption satn.. In the presence of barium sulfate, carbon, and alumina, two types in the isotherms are obsd. Adsorption of BSA is affected by changes in the pH, ionic strength, and temp. of the medium. In the presence of metallic chromium, adsorbed BSA mols. are either denatured or neg. adsorbed at the metallic interface.

Due to the presence of pores in ion-exchange resins, adsorption of BSA is followed by preferential hydration on the resin surfaces in some cases. Sometimes 2 steps of isotherms are also obsd. during adsorption of BSA on the solid resins in chloride form. Adsorption of BSA, .beta.-lactoglobulin, gelatin, myosin, and lysozyme is neg. on Sephadex surface due to the excess adsorption of water by Sephadex. The neg. adsorption is significantly affected in the presence of CaCl<sub>2</sub>, KSCN, LiCl, Na<sub>2</sub>SO<sub>4</sub>, NaI, KCl, and urea. The abs. amts. of water

and protein simultaneously adsorbed on the surface of different solids have been evaluated in some cases by crit. thermodn. anal. The std. free energies (.DELTA.G<sub>0</sub>) of excess pos. and neg. adsorption of the protein per

square meter at monolayer satn. were calcd. using a proposed universal scale of thermodn. The free energy of adsorption with ref. to this state is strictly comparable to each other. The magnitude of std. free energy of transfer (.DELTA.G<sub>B0</sub>) of one mole of protein or a protein mixt. at any type of physicochem. condition and at any type of surface is 38.5 kJ/mol.

L23 ANSWER 8 OF 13 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

AN 1990-257610 [34] WPIDS

AB JP 02180899 A UPAB: 19930928

Purificn. of immunoglobulin is carried out by chromatography with anion exchanger contg. diethylamino gp.. After contact with the anion exchanger, the sample is developed with first buffer contg. 100 mM - 130 mM NaCl. The immunoglobulin is then eluted with 2nd buffer which contains

higher concn. than 1st and below 160 mM NaCl.

USE/ADVANTAGE - By this invention, immunoglobulin can be purified highly by simple method. Immunoglobulin can be purified to give a single band in SDS-polyacrylamide gel electrophoresis. The method has higher operation efficiency than by antigen -specific antibody purifcn. method.

In an example, mouse hybridoma which produces monoclonal antibody (IgM) against colic cancer, was implanted. After 10 days, immunoglobulin-contg. ascites fluid (ca. 5ml) was obtd.. 100% satd.  $(\text{NH}_4)_2\text{SO}_4$  aq. soln. (5ml) was added to the fluid, dropped on ice cooling, and ppte. was collected by centrifugation. The ppte. was dissolved in

100

mM NaCl 20 mM Tris-HCl, buffer (pH 8.5; 10ml), and dialysed at 4 deg.C overnight with the same buffer. The absorbence of the obtd.

antibody fraction at 280 nm was 5.41. The antibody fraction (5ml) was applied to DEAE-Sephadex column equilibrated with the same buffer, and eluted with 20 mM Tris-HCl buffers contg. 100 mM, 150

mM

and 1000 mM NaCl in order. Elution pattern of each fractions were assayed

at 280 nm absorbence. Second peak was that of immunoglobulin. 0/0

L23 ANSWER 9 OF 13 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD  
AN 1988-086199 [13] WPIDS

AB EP 261941 A UPAB: 19930923

Purified tissue plasminogen activator (tPA) having a molecular wt, of about 70000 daltons is new.

In the prepn., a crude tPA prep. contg. protein impurities of molecular wts. other than tPA of molecular wt. 70000 is contacted with a cation exchange resin, and the tPA is selectively sepd. by salt gradient elution. The resin pref. has a carboxymethyl, phosphato or sulphopropyl gp. Elution is pref. at pH 6-7.5. The salt used is esp. NaCl, KCl, Na or K phosphates etc.

USE/ADVANTAGE - The tPA is obtd. selectively from prote proteins reacting with antihuman tPA antibody and having different molecular wt. It is useful for the treatment of thrombosis.

0/0

ABEQ EP 261941 B UPAB: 19930923

A process for purifying tPA comprising the steps of: (a) contacting a carboxymethyl agarose exchange resin with a crude tPA preparation containing tPA having a molecular weight of about 70,000 daltons, together

with impure tPA species including active degraded products of tPA, polymers of tPA, and complexes of tPA with other proteins, said impure

tPA

species having molecular weights other than about 70,000 daltons and capable of reacting with an anti-human tPA antibody: (b) treating said exchange resin with an eluant having a salt concentration

of

less than 0.15M and a pH in the range of 6.0 - 6.4 to elute the impure

tPA

species having molecular weights of less than about 70,000 daltons; and thereafter (c) treating said exchange resin with an eluant having a salt concentration in the range of 0.175 - 0.375M and a pH in the range of 6.0 - 6.4 to elute the tPA having a molecular weight of about 70,000 daltons and recovering the resultant eluate.

0/0

ABEQ US 4985362 A UPAB: 19930923

Tissue plasminogen activator (TPA) is purified by A) contacting a carboxymethyl agarose exchange resin (ER) with crude TPA contg. TPAe of

mol wt. 70 kD and other TPA moieties of mol.wt. other than 70 kD and able to react with an anti-human TPA antibody, B) eluting the ER with an eluant contg. less than 0.15 M salt and having a pH 6.0-6.4 to elute impure TPA moieties of mol.wt. below 70 kD and C) eluting the ER with an eluent contg. 0.175-0.376N salt and having a pH 6.0-6.4 to eluate TPA of mol.wt. 70 kD and recovering the purified TPA.

ADVANTAGE - An effective way to purify TPA of mol.wt. 70 kD.

L23 ANSWER 10 OF 13 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

AN 1984-158915 [26] WPIDS

AB CA 1168152 A UPAB: 19930925

The prodn. comprises treatment of a dil. soln. contg. IgG with NaCl and glycine, followed by ultrafiltration to give a concd. soln. It may be freeze dried.

The starting soln. may be an eluate obtd. from a column of ion-exchange resin e.g. DEAE-"Sephadex" A-50

(RTM). Human plasma may be diluted with water then applied to such a column, and elutionis with 0.025M phosphate buffer at pH 7-5.

The prods. are used for intravenous injection. The freeze dried solid

prods. are reconstituted for use. The plasma used may be normal or it may have a high concn. of antibodies, esp. Rh factor antibodies, rabies antibodies, tetanus antibodies or zoster antibodies, depending on the use intended. The prods. have good stability.

0/0

L23 ANSWER 11 OF 13 MEDLINE

DUPLICATE 3

78041676 Document Number: 78041676. Chemical and biological properties of B16 murine melanoma cells grown in defined medium containing bovine serum albumin. Banks J R; Bhavanandan V P; Davidson E A. CANCER RESEARCH, (1977 Dec) 37 (12) 4336-45. Journal code: CNF. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB The addition of 1 percent (w/v) bovine serum albumin (BSA) to the F12 medium utilized for the growth of the B16 melanoma cells significantly stimulated the growth of this cell line. The synthesis of mucopolysaccharides and sialoglycopeptides in this medium is identical with that in Eagle's minimal essential medium with Earle's balanced salt solution supplemented with 2 mM L-glutamine, twice the recommended concentration of vitamins, nonessential amino acids, sodium pyruvate, and 10 percent (v/v) fetal calf serum. Cell volume and morphology did not change significantly, under the different growth conditions and tumorigenicity, as assayed by injection of cultured cells into syngeneic animals, was not decreased. Analysis of the BSA used indicated the presence of a sialoglycoprotein contaminant. This sialoglycoprotein contaminant was present in all lots examined and contains N-acetyl-and N-glycolylneuraminic acid, mannose, galactose, and glucosamine. The sialoglycoprotein can be removed by chromatography on acetate form anion-exchange resin at pH 4.3. F12 media containing the purified BSA plus selenite and the sodium salts of palmitic, oleic, and linoleic acids supported growth of the melanoma cells to the same extent as did the media containing unpurified BSA, indicating that the sialoglycoprotein has no role in sustaining the growth of the cells.

L23 ANSWER 12 OF 13 MEDLINE

73155754 Document Number: 73155754. Determination of gastrin in serum. An evaluation of the reliability of a radioimmunoassay. Stadil F; Rehfeld J F. SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, (1973) 8 (2) 101-12.

Journal

code: UCS. ISSN: 0036-5521. Pub. country: Norway. Language: English.

L23 ANSWER 13 OF 13 MEDLINE  
70240760 Document Number: 70240760. [Purification of hemoglobin contaminated albumin solutions by means of zinc salts]. Purification par les sels de zinc des solutions d'albumine souillées par l'hémoglobine. Mouray H; Heron de Villefosse M; Crouzat-Reynes G. REVUE FRANCAISE DE TRANSFUSION, (1969 Dec) 12 (4) 469-75. Journal code: S1E. ISSN: 0035-2977. Pub. country: France. Language: French.

L24 40 FILE MEDLINE  
L25 89 FILE CAPLUS  
L26 27 FILE BIOSIS  
L27 27 FILE EMBASE  
L28 47 FILE WPIDS

TOTAL FOR ALL FILES  
L29 230 L16 AND SEPARAT?

=> s (dimer or multimer) and 129  
L30 0 FILE MEDLINE  
L31 0 FILE CAPLUS  
L32 0 FILE BIOSIS  
L33 0 FILE EMBASE  
L34 2 FILE WPIDS

TOTAL FOR ALL FILES  
L35 2 (DIMER OR MULTIMER) AND L29

=> d 1-2

L35 ANSWER 1 OF 2 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD  
AN 1996-464965 [46] WPIDS  
DNC C1996-146030  
TI Modified obese (ob) gene prods. - comprising ob **dimers**, ob **dimer** fusion proteins and ob monomer fusion proteins, useful, e.g. for treating obesity or diabetes.  
DC A96 B04 D16 J04  
IN ALBRANDT, K A; BEELEY, N; BEIDLER, D E; CHUN, M; JANES, S M; PARK, D M; PHELPS, J L; PRICKETT, K S; RINK, T J; SIERZEGA, M E  
PA (AMYL-N) AMYLIN PHARM INC  
CYC 65  
PI WO 9631526 A1 19961010 (199646)\* EN 130p C07K001-00  
RW: AT BE CH DE DK ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE  
SZ UG  
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE  
KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE  
SG SI SK TJ TM TT UA UG US UZ VN  
AU 9655395 A 19961023 (199707) C07K001-00  
ADT WO 9631526 A1 WO 1996-US4909 19960405; AU 9655395 A AU 1996-55395  
19960405  
FDT AU 9655395 A Based on WO 9631526  
PRAI US 1995-419598 19950406  
IC ICM C07K001-00  
ICS A01N037-18; A61K038-00; C07H019-00; C07H021-00; C07H021-02;  
C07H021-04; C07K014-00; C07K017-00; C12N001-20; C12N005-00;  
C12N015-00; C12N015-09; C12N015-63; C12N015-70; C12N015-74;  
C12P019-34; C12P021-06; C12Q001-68

L35 ANSWER 2 OF 2 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD  
AN 1991-078673 [11] WPIDS  
DNC C1991-033646  
TI Prodn. of human interleukin 5 in high yields - includes adding ammonium sulphate to supernatant contg. interleukin 5 and purifying obtd. ppte. by chromatography.  
DC B04 D16  
PA (SUNR) SUNTORY LTD  
CYC 1  
PI JP 03027295 A 19910205 (199111)\* 13p  
JP 2813370 B2 19981022 (199847) 11p C12P021-02  
ADT JP 03027295 A JP 1989-160188 19890622; JP 2813370 B2 JP 1989-160188 19890622  
FDT JP 2813370 B2 Previous Publ. JP 03027295  
PRAI JP 1989-160188 19890622  
IC C07K003-20; C12N015-24; C12P021-02; C12R001-91  
ICM C12P021-02  
ICS C07K001-36; C07K003-20; C07K014-54; C12N015-09; C12N015-24;  
C12R001-91  
ICI C12P021-02, C12R001:91

=> s chromatograph? and 129  
L36 27 FILE MEDLINE  
L37 44 FILE CAPLUS  
L38 16 FILE BIOSIS  
L39 14 FILE EMBASE  
L40 19 FILE WPIDS

TOTAL FOR ALL FILES  
L41 120 CHROMATOGRAPH? AND L29

=> s (gradient or linear or stepwise) and 141  
L42 9 FILE MEDLINE  
L43 8 FILE CAPLUS  
L44 1 FILE BIOSIS  
L45 4 FILE EMBASE  
L46 3 FILE WPIDS

TOTAL FOR ALL FILES  
L47 25 (GRADIENT OR LINEAR OR STEPWISE) AND L41

=> dup rem 147  
PROCESSING COMPLETED FOR L47  
L48 20 DUP REM L47 (5 DUPLICATES REMOVED)

=> d 1-20 cbib abs;s (alpha 1 integrin or lfa 1alpha or beta 2 integrin or b1 or bp35) and 116

L48 ANSWER 1 OF 20 MEDLINE DUPLICATE 1  
1999026968 Document Number: 99026968. Isolation and preliminary characterization of histone H1.b allelic variants from quail erythrocytes.  
Palyga J; Neelin J M. (Department of Genetics, Wyższa Szkoła Pedagogiczna,  
Kielce, Poland.) GENOME, (1998 Oct) 41 (5) 709-19. Journal code: FNP.  
ISSN: 0831-2796. Pub. country: Canada. Language: English.  
AB Our goal was to purify and characterize the allelic variants H1b1 and H1b2  
of histone H1.b, one of the seven subtypes of this linker histone

extracted from Japanese quail erythrocyte nuclei. These variants are revealed phenotypically as band H1.3 or part of band H1.4 by polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate (SDS).

All H1 subtypes together were separated from H5 by gel-permeation chromatography through Bio-Gel P-150. H1 was then fractionated on a column of the cation-exchange resin Amberlite CG-50 by using a shallow guanidine hydrochloride gradient, which enriched subtype H1.b together with H1.z and overlapping with subtypes H1.a and H1.b. Alternatively purification of subtypes was achieved electrophoretically: total H1 fractions from quail with different H1 phenotypes were first resolved into sub-types by PAGE

in

acetic acid-urea; after staining, the appropriate H1.b bands from several parallel gel pieces were excised and the histone was concentrated by PAGE in SDS. After fragmentation of H1.b in the gel pieces with N-bromosuccinimide (NBS), PAGE in SDS indicated no difference between

H1b1

and H1b2 in the C-terminal "half" of the polypeptides. In contrast, limited digestion with endoprotease V8 from *Staphylococcus aureus* has shown that differences, probably by a few residues in length, reside in the N-terminal part of the molecule, close to the amino-terminus.

L48 ANSWER 2 OF 20 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

1998056203 EMBASE Bovine whey fractionation based on cation-exchange chromatography. Hahn R.; Schulz P.M.; Schaupp C.; Jungbauer A.. A. Jungbauer, Institute of Applied Microbiology, University of Agriculture, Muthgasse 18, A-1190 Vienna, Austria. Journal of Chromatography A 795/2 (277-287) 6 Feb 1998.

Refs: 29.

ISSN: 0021-9673. CODEN: JCRAEY.  
Publisher Ident.: S 0021-9673(97)01030-3. Pub. Country: Netherlands.  
Language: English. Summary Language: English.

AB Bovine whey proteins have potential applications in veterinary medicine, food industry and as supplements for cell culture media. A fractionation scheme for the economically interesting proteins, such as IgG,

lactoferrin

and lactoperoxidase, based on cation exchangers was the goal of our investigations. A chromatographic process was developed where .alpha.-lactalbumin passes through the column and separation of the desired proteins is achieved. Four different cation-exchange media (S-HyperD-F, S-Sepharose FF, Fractogel EMD SO3- 650 (S) and Macro-Prep High S Support) were compared in regard to their dynamic binding capacity for IgG and their different elution behaviours when sequential step gradients with NaCl buffers were applied. Peak fractions were analyzed by size-exclusion chromatography and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Lactoperoxidase activity was monitored by the oxidation of o-phenylenediamine. In order to explain the different resolution behaviours, isocratic runs with pure standards of whey proteins were performed. The k' values were calculated and plotted against salt concentration. Fractogel EMD had the highest binding capacity

for IgG, 3.7 mg/ml gel at a linear flow-rate of 100 cm/h, but the resolution was low compared to that with the other three media. S-Hyper D and S-Sepharose FF showed lower capacities, 3.3 and 3.2 mg/ml gel, respectively, but exhibited better protein resolution. These effects could be partially explained by the k' versus salt concentration plots. The binding capacity of Macro-Prep S was considerably lower compared to that of the other resins investigated because its selectivity for whey proteins was completely different. S-Sepharose FF and S-Hyper D combine

relatively high dynamic capacity for IgG and good resolution. Compared to studies with standard proteins, such as 100 mg/ml bovine serum albumin for S-Hyper D, their binding capacities were very low. Even after removal of low-molecular-mass compounds, the capacity could not be improved significantly. The running conditions (low pH) were responsible for the low protein binding capacity, since low-molecular-mass compounds in the feed do not compete with the adsorption of whey protein. The dynamic capacity did not decrease to a large extent within the range of flow-rates (100-600 cm/h) investigated. The dynamic capacity of HyperD and Fractogel was at least five times higher when pure bovine IgG was used for determination. In conclusion, S-Sepharose FF, S-Hyper D-F and Fractogel EMD SO3- 650 (S) are considered as successful candidates for the large-scale purification of bovine whey proteins.

L48 ANSWER 3 OF 20 MEDLINE

96414879 Document Number: 96414879. Influence of column type and chromatographic conditions on the ion-exchange chromatography of immunoglobulins. Yang Y B; Harrison K. (Separations Group, Inc., Hesperia, CA 92345, USA. )JOURNAL OF CHROMATOGRAPHY. A, (1996 Aug 30) 743 (1) 171-80. Journal code: BXJ. Pub. country: Netherlands. Language: English.

AB Immunoglobulins are often purified by affinity chromatography. However, this technique is costly, can result in poor resolution for subclasses (or is only group specific), and leads to possible leaching of contaminants into the purified products. Ion-exchange chromatography has shown great potential and has found an increased usage in the purification of immunoglobulins. The aim of this study is to further understand the separation mechanism with emphasis on the influence of column type and chromatographic conditions on the peak shape, selectivity and changes in the elution patterns. Included are strong cation-exchange, strong anion-exchange and weak anion-exchange columns. Five immunoglobulin G antibodies were used as test probes. Some sera and ascites were also used in the study. Among the chromatographic conditions examined were mobile phase pH, buffer type, buffer concentration, gradient rate, and column temperature. Significant differences in the chromatographic behavior (elution pattern, peak shape and selectivity) of the test samples are discussed in regard to the column type and the chromatographic conditions.

L48 ANSWER 4 OF 20 MEDLINE

96414878 Document Number: 96414878. Rapid purification and monitoring of immunoglobulin M from ascites by perfusion ion-exchange chromatography. McCarthy E; Vella G; Mhatre R; Lim Y P. (PerSeptive Biosystems, Inc., Framingham, MA 01701, USA. )JOURNAL OF CHROMATOGRAPHY. A, (1996 Aug 30) 743 (1) 163-70. Journal code: BXJ. Pub. country: Netherlands. Language: English.

AB A purification and on-line monitoring procedure for IgM was developed. Perfusion ion-exchange chromatography was used for rapid purification of IgM from ascites fluid and hybridoma supernatant. Crude ascites was directly loaded onto an ion exchanger. Due to the complexity of IgM, a two-step ion-exchange procedure had to be developed. This procedure involved a rapid cation-exchange chromatography capture step followed by further purification using anion-exchange chromatography. High linear velocities, in excess of 3500 cm/h, enabled separations to be performed under 5 min.

Purity of the final product by SDS-PAGE was shown to be greater than 95%. Furthermore, the **antibodies** retained biological activity as measured by indirect immunofluorescence (IIF) and ELISA. The IgM peak was also monitored on-line using a novel peak tracking approach. This involved

placing an **antibody** column (specific to the IgM) prior to the ion-exchange column and operating the ion-exchange column with and without

the **antibody** column in-line. The missing peak that is identified by comparing the two chromatograms indicates where the IgM elutes.

L48 ANSWER 5 OF 20 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD  
AN 1994-200248 [24] WPIDS  
AB WO 9412618 A UPAB: 19940803

A purified heparinase II isolated from *Flavobacterium heparinum* (Fh) free of lyase activity other than heparinase II activity is claimed.

Also claimed are: a purified heparinase III isolated from Fh free of lyase activity other than heparinase III activity; a method for purifying heparinase I, II and III from Fh comprising (a) lysing Fh cells in a pure culture of Fh, (b) removing cell debris and nucleic acids from the cell lysate, (c) absorption of heparinase I, II and III, to hydroxyapatite,

(d) absorption of non-heparinase I, II and III proteins to GAE-resin, (e) **sepn.** of heparinase I, II and III by HPLC on a hydroxyapatite column, (f) purificn. of the **sepd.** heparinases by cation exchange FPLC and (g) purificn. of the **sepd.** heparinases by gel permeation HPLC; a monoclonal **antibody** (MAb) cross-reactive with differential affinities for heparinase I, II and III from Fh; a method

for detecting heparinase comprising reacting a sample having heparinase activity with an **antibody** cross-reactive with differential affinities for heparinase I, II and III from Fh and determining the extent

of the reaction; and a method for cleaving heparin and heparin sulphate comprising reacting heparin or heparin sulphate with a purified heparinase

selected from heparinase I, II and III from Fh not contaminated with any lyases.

USE - The purified heparinases can be used for the depolymerisation of heparin and heparin sulphate for structure and activity studies and also in clinical studies. The **antibodies** can be used for isolation, detection and characterisation of the heparinases and in studies involving substrate specificity, enzyme inhibition and active site

mapping.

Dwg.0/6

ABEQ US 5389539 A UPAB: 19950328

Heparinases-I, -II and -III have been isolated from cultures of *Flavobacterium heparinum* and purified by selective **chromatographic** adsorption, removing other proteins by adsorption on QAE-resin, isolation of the heparinases by selective adsorption on hydroxyapatite, and **sepn.** of the three heparinases using a **cation exchange resin** and gel permeation **chromatography**.

. Heparinases-I, -II and -III have Mr 42,800; 84,100 and 70,800 respectively; heparinase-III cleaves heparan sulphate selectively

(optimum

pH 9.9-10.1), and heparinase-II cleaves both heparin and heparan sulphate (optimum pH 8.9-9.1), and the enzymes are free of other lyase activity.

USE - The enzymes facilitate selective cleavage of heparin and heparan sulphate, and are also antigens for the prodn. of corresp. **antibodies** for the purification and characterisation of the

enzymes.

ADVANTAGE - The prods. exhibit improved heparinase selectivity.  
Dwg.0/0

ABEQ US 5569600 A UPAB: 19961205

Cleaving hexosamine-glucuronic acid linkages in linear polysaccharides of D-glucosamine linked to hexuronic acid comprises reacting heparin or heparan sulphate with a purified heparinase selected from heparinase II present in Flavobacterium heparinum free of lyase activity other than heparinase II activity, having mol. wt. 84100, cleaving heparin and heparan sulphate and having pH optimum of 8.9-9.1

and

heparinase III which is expressed in Flavobacterium heparinum free of lyase activity other than heparinase III activity, having mol. wt. 70800, cleaving heparan sulphate and having a pH optimum of 9.9-10.1.

Dwg.0/6

L48 ANSWER 6 OF 20 MEDLINE

94282267 Document Number: 94282267. Separation of closely related intrinsic membrane polypeptides of the photosystem II light-harvesting complex (LHC II) by reversed-phase high-performance liquid chromatography on a poly(styrene-divinylbenzene) column. Damm I; Green B R. (Botany Department, University of British Columbia, Vancouver, Canada..) JOURNAL OF CHROMATOGRAPHY. A, (1994 Mar 25) 664 (1) 33-8. Journal code: BXJ. Pub. country: Netherlands. Language: English.

AB The three closely related intrinsic membrane polypeptides of the photosystem II light-harvesting complex (LHC II) were successfully resolved on a PRP-1 poly(styrene-divinylbenzene) column using a three-stage linear water-acetonitrile gradient containing 0.1% trifluoroacetic acid. The hydrophobic proteins of photosystem I (PS I-200) and photosystem II core particles were also separated by this method, showing that membrane proteins of different sizes and hydrophobicities can be resolved in this system.

L48 ANSWER 7 OF 20 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

AN 1994-007528 [01] WPIDS

AB WO 9325670 A UPAB: 19940217

Process (I) comprises sepn. deamidated and non-deamidated human DNase I from a mixt..

Also claimed are: (1) purified deamidated human DNase; (2) purified non-deamidated human DNase; (3) a pharmaceutical compsn. consisting of

the

deamidated human DNase and opt. a pharmaceutically acceptable excipient; (4) a pharmaceutical compsn. consisting on non-deamidated human DNase and opt. a pharmaceutically acceptable excipient; (5) a pharmaceutical compsn.

comprising non-deamidated human DNase in a plastic vial; and (6) storage of human DNase by preparing a compsn. comprising non-deamidated human DNase in an aq. soln. having pH 4.5 - 6.8 and storing the compsn. for greater than 3 weeks.

Sepn. process requires a tentacle cation exchange resin, an immobilised heparin resin and an immobilised non-hydrolysable DNA analogue resin.

USE/ADVANTAGE - Deamidated human DNase or non-deamidated human DNase may be used for treatment of patients having an accumulation of viscous, DNA-contg. material. Admin. of purified DNases pref. is via direct inhalation into the lungs. Non-deamidated human DNase may be admin. directly into the airway passages for the treatment of patients having pulmonary diseases e.g. chronic bronchitis, cystic fibrosis or emphysema. The DNases are employed for enzymatic alteration of the viscoelasticity

of

mucous, for treatment of patients with abnormal viscous, purulent

secretions e.g. with infectious pneumonia, asthma, tuberculosis and fungal infections.

In an example, purified deamidated human DNase and purified non-deamidated human DNase for use in this study were prep'd. by TCX chromatography. DNase enzymatic activity, synthetic double stranded DNA, 25 base pairs in length, was labelled with dinitrophenol (DNP) on one end and with biotin on the other end. Hydrolysis of the substrate by DNase was detected by capture of the reaction prods. on microtiter plate wells coated with antibody to DNP and by quantitation of the intact probe with streptavidin-horseradish peroxidase.

Specific activity of stability samples was correlated ( $r^2 = 0.613$ ;

$n=5$ ) with the extent of DNase deamidation (range 27% - 93%).

Extrapolation

of the least squares linear equation provided an estimate that the specific activity of deamidated human DNase was approx. 77% lower than

that of non-deamidated human DNase.

Dwg.0/9

ABEQ US 5279823 A UPAB: 19940307

Deamidated and non-deamidated human DNases have been prep'd. by recombinant

DNA methods and sepd. and purified by chromatography with a heparin or non-hydrolysable DNA analogue bonded to a resin or other

support medium as adsorbent. These enzymes are phosphodiesterases that cleave polydeoxyribonucleic acids. Pharmaceutical compsns. contg. deamidated or non-deamidated DNase and the usual carriers and additives reduce the viscoelasticity of pulmonary secretions.

USE/ADVANTAGE - The prods. are therapeutics for chronic bronchitis, cystic fibrosis, emphysema, etc. The recombinant enzymes are free from contamination with proteases and other proteins.

Dwg.0/9

ABEQ GB 2282140 B UPAB: 19960510

A process comprising separating deamidated and non-deamidated human DNase from a mixture thereof.

L48 ANSWER 8 OF 20 MEDLINE

DUPLICATE 2

92147812 Document Number: 92147812. Immobilized metal affinity chromatography as a means of fractionating microsomal cytochrome P-450 isozymes. Roos P H. (Institut fur Physiologische Chemie I, Abteilung

Bioenergetik, Ruhr-Universitat Bochum, Germany.. )JOURNAL OF CHROMATOGRAPHY, (1991 Nov 29) 587 (1) 33-42. Journal code: HQF. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB Fractionation of microsomal cytochrome P-450s is usually done by chromatography on ion-exchange resins and hydroxyapatite. The resolution of the great number of similar P-450 isozymes, however, requires additional methods based on different separation parameters. For this purpose immobilized-metal affinity chromatography (IMAC) was applied to the separation of P-450 isozymes. The method in its application to rat liver microsomes is described in detail. For method optimization and for the reproducibility of analytical fractionations a completely automatic fast protein liquid chromatographic system especially designed for IMAC is presented. Optimization is done with respect to the choice of the immobilized metal ion and the elution conditions. The chromatographic resolution is markedly enhanced by using segmented vs. linear gradients. The efficiency of P-450 resolution is demonstrated by

sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting, verifying the different retention behaviours of the isozymes. However, for all the isozymes analysed so far, reactivity with one particular polyclonal antibody is observed with more than two IMAC fractions of a single run. This may be explained in part by the occurrence of isozymic forms distinguishable by the pattern of chymotryptic peptides. Hence IMAC appears to be suitable for the separation of closely related isozyme forms.

- L48 ANSWER 9 OF 20 CAPLUS COPYRIGHT 1999 ACS  
1987:571985 Document No. 107:171985 Isolation of .alpha.2-globulin associated with Crohn's disease. Chekhonin, V. P.; Khalif, I. L.; Kirkin, B. V.; Ovchinnikov, A. V. (Scientific-Research Institute of Proctology, USSR). U.S.S.R. SU 1318915 A1 19870623 From: Otkrytiya, Izobret. 1987, (23), 157-8. (Russian). CODEN: URXXAF. APPLICATION: SU 1985-3973473 19851118.
- AB .alpha.2-Globulin assocd. with Crohn's disease is isolated from affected tissue of the gastrointestinal tract by extn. of homogenated tissue with detergents in pH 8.6 buffer, salting-out, and chromatog. on an anion exchange resin eluted with 0.25-0.5 M linear gradient NaCl. The fractions eluting with 0.38-0.46 M NaCl were further sepd. by isoelec. chromatog. pH 4.8-5.6 and gel filtration. .alpha.2-Globulin was purified from an 85,000 +- 10 mol. wt. fraction by affinity chromatog. using antibody immunosorbent and elution with 0.02 M buffer pH 2.2.

- L48 ANSWER 10 OF 20 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD  
AN 1986-320384 [49] WPIDS  
AB EP 203382 A UPAB: 19930922
- Prodn. of recombinant alpha-interferon (I) comprises cultivating a host organism, pref. E.coli, which contains a (I)-coding gene, then after a specified culture time, the cells are killed, esp. lysed in a homogeniser at pH2. Expressed (I) is sepd. conventionally, the cell debris removed and (I) conc. and pre-purified by tandem chromatography. The eluate is adjusted to pH 4-4.8 to separate impurities, then (I) finally purified by chromatography on a cationic exchange column, pref. MONO-S, type HR 10/10, eluting with a volatile buffer (esp. a linear gradient of 0.1-0.5 M NH4 acetate of pH 4-5, esp. 4.5). The purified product is then lyophilised.

The cells are harvested at a time when not over 20%, esp. not over 1%, methionine-interferon has been formed. The gene pref. codes for the human interferon sequence (Ia).

CysAspLeuPro GlnThrHisSer LeuGlySerArg ArgThrLeuMet LeuLeuAla GlnMetArg ArgIleSerLeu PheSerCysLeu LysAspArgArg AspPheGlyPhePro GlnGluGluPhe GlyAsnGlnPhe GlnLysAlaGluThr IleProValLeuHis GluMetIleGlu GlnIlePheAsn LeuPheSerThrLysAspSer SerAlaAlaTrpAsp GluThrLeuLeuAsp LysPheTyrThrGlu LeuTyrGlnGlnLeuAsn AspLeuGluAla CysValIleGln GlyValGlyValThrGlu ThrProLeuMet LysGluAspSer IleLeuAlaVal ArgLysTyrPheGlu ArgIleThrLeuTyr LeuLysGluLysLysTyrSer ProCysAlaTrpGlu ValValArgAlaGlu IleMetArgSerPhe SerLeuSerThrAsnLeu GlnGluSerLeu ArgSerLysGlu (Ia).

(Ia) in pure, homogeneous form is itself claimed. It contains less than 5% methionine-interferon; less than 0.2% oligomer; less than 2% di-, tri- or tetra-mers; is free of reduced forms and fragments, and more than 90% of the monomer present is native alpha-interferon with disulphide bridged between Cys residues 1/98 and 29/138.

USE/ADVANTAGE - (I) is useful for treating viral infections and tumours. It can be prep'd. in highly pure, non-immunogenic form.

0/13

ABEQ US 5196323 A UPAB: 19930922

Process for prepn. and purifcn. of recombinant interferon-alpha comprises:- (a) cultivating E.coli contg. the IFN gene so that not more than 5% Met-IFN is formed. (b) extracting and concentrating the interferon. (c) subjecting the IFN to tandem **chromatography**, comprising **sepn.** on a cellulase column followed by an anti-alpha-IFN monodonal **antibody** column; (d) subjecting this to isoelectric precipn. of impurities at pH 4.0-4.8; and (e) purifying IFN by

**chromatog.** on a high performance cation exchange column using a volatile buffer.

USE/ADVANTAGE - IFN is non-immunogenic on parenteral admin. Is pure, virtually free from endotoxins and is used as an antiviral and immunoregulatory agent.

0/13

ABEQ EP 203382 B UPAB: 19931123

Process for the preparation of recombinant alpha-interferon, characterised

in that the host organism containing the interferon gene is cultivated under conventional conditions, after a growth period in which not more than 20% methionine interferon is formed the cells are killed off and harvested, the expressed interferon is removed in conventional manner,

the

cell debris is removed in a slightly alkaline medium, the interferon is concentrated and subjected to preliminary purification by tandem **chromatography**, the eluate is adjusted to pH 4.0-4.8 to remove any impurities, the interferon is finally purified by **chromatography** on a cation exchanger column with a volatile buffer as eluant at pH levels

from 4.0 to 5.0 and is then lyophilised.

7

Dwg.0/4

ABEQ DE 3515336 C UPAB: 19940303

Prodn. of alpha-interferon comprises transforming host cells, e.g., E. coli, with an expression vector contg. a DNA sequence that encodes the formation of alpha-interferon; then propagation of the transformed cells to produce the exogenous **polypeptide** until not more than 20% of the prod. contains methionine-interferon; the cells are then disintegrated

and extracted with very dil. aq. alkali; the extract is concd. and purified by cellulose column **chromatography** and affinity **chromatography** (using an immobilised highly specific monoclonal **antibody**); the pH of the eluate is adjusted to 4.0-4.8 and the soln. is passed through a column of **cation exchange resin**; and then freeze-dried. The aminoacid sequence of the interferone is given.

USE - Pharmaceutical compsns. contg. alpha-interferon and opt. other active components are therapeutics for viral infections, cancer, immunoregulatory disorders, etc..

Dwg.0/12

L48 ANSWER 11 OF 20 MEDLINE

86278561 Document Number: 86278561. Comparison of porous silica packing materials for preparative ion-exchange **chromatography**. Schmuck M N; Gooding D L; Gooding K M. JOURNAL OF CHROMATOGRAPHY, (1986 May 30) 359 323-30. Journal code: HQF. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB Although analytical high-performance liquid **chromatographic** columns have been successfully used for purification of milligram amounts of proteins, they do not appear to be ideal for preparing gram or kilogram quantities because of cost and load capacities. In this paper the

development of preparative weak anion-exchange materials is described. These materials possess similar chromatographic characteristics to analytical 5-10 micron materials, yet also have high load capacities.

A

number of inorganic packings were examined to determine which had the best combination of high load capacity, good resolution, stability, and low cost. When appropriate flow-rates and gradient shapes were used, 30-50 micron materials produced resolution of components of a commercial ovalbumin sample that was comparable to that achieved on a 6-micron material. An amount of 3 g of a protein could be loaded onto a 250 X 21 mm-I.D. column with adequate resolution to separate it from some of its impurities.

L48 ANSWER 12 OF 20 MEDLINE

85030709 Document Number: 85030709. Fractionation of human red cell membrane

proteins by ion-exchange chromatography in detergent on Mono Q, with special reference to the glucose transporter. Lundahl P; Greijer E; Lindblom H; Fagerstam L G. JOURNAL OF CHROMATOGRAPHY, (1984 Aug 3) 297 129-37. Journal code: HQF. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB The anion exchanger Mono Q has been used for rapid and efficient fractionation of human red cell membrane proteins in the easily removable detergents n-octyl-beta-D-glucopyranoside or nonanoyl-N-methylglucamide. In practice the chromatographic resolution of membrane proteins was lower than for water-soluble proteins, perhaps due to protein-protein interactions and microheterogeneity, but several components, or groups of components, separated well upon salt gradient elution. The glucose transporter (or transportase) was eluted early, glycophorins later, and the anion transporter still later. The detergents Berol 185

and

the zwitter-ionic derivatives of cholate, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate and 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulphonate, gave similar chromatographic results but differed in solubilization selectivity. A relatively pure material was also fractionated; viz., a glucose transportase which had been prepared by DEAE-cellulose chromatography. Mono Q, in the presence of octyl glucoside, afforded additional purification, which made automatic sequence

determination possible for eighteen amino acid residues. The results indicate that two polypeptides were present in about equimolar amounts.

L48 ANSWER 13 OF 20 CAPLUS COPYRIGHT 1999 ACS

1979:99386 Document No. 90:99386 RNA hybridization to DNA coupled with cyanogen-bromide-activated Sephadex. The purification of polyoma messenger RNA. Siddell, Stuart G. (Transl. Lab., Imp. Cancer Res. Fund, London, Engl.). Eur. J. Biochem., 92(2), 621-9 (English) 1978. CODEN: EJBCAI. ISSN: 0014-2956.

AB Denatured DNA can be immobilized on CNBr-activated Sephadex G 10 while remaining freely available for hybridization. However, after activation with CNBr followed by the coupling of DNA and deactivation with ethanolamine, Sephadex acts as an ion-exchange resin. It is therefore necessary to elute RNA from DNA-Sephadex by a combination of formamide buffers contg. NaCl and elevated temps., conditions which dissoc. DNA-RNA hybrids and also prevent the binding of RNA to the resin by ion exchange. Polyoma DNA, or restriction fragments thereof, were bound to Sephadex and used to purify viral mRNA. RNA purified from polyadenylated RNA isolated from the cytoplasm of mouse

cells 28-30 h after infection with polyoma virus sedimented at values between 19 and 16 S in sucrose/formamide gradients and, in a wheat germ-free translation system, directed the synthesis of 3 polypeptides; these were identified by gel mobility, immunopptn., and fingerprinting as the 3 structural proteins of polyoma virus. Viral mRNA could also be purified by hybridization directly from total cytoplasmic infected-cell RNA without prior selection of poly(A)-contg. RNA. The advantages and limitations of DNA attached to CNBr-activated Sephadex as an affinity matrix for RNA purifn. are discussed.

L48 ANSWER 14 OF 20 CAPLUS COPYRIGHT 1999 ACS  
1977:187444 Document No. 86:187444 Isolation from the thymus and study of the nature of the factor stimulating immunogenesis. Morozov, V. G.; Khavinson, V. Kh.; Pisarev, O. A. (Leningr. Gos. Inst. Usoversh. Vrachei im. Kirova, Leningrad, USSR). Dokl. Akad. Nauk SSSR, 233(3), 491-4 [Physiol.] (Russian) 1977. CODEN: DANKAS.

AB The crude prepn. of thymarin from calf thymus was sepd. into 3 fractions by chromatog. on carboxyl-contg. cation exchange resin by elution with continuous gradient of pH 3.2-10.5. The immunostimulating activity was concd. 50-100-fold in fraction 3 of basic character, mol. wt. 2000-5000 daltons, contg. 8 different amino acids. Administration of 0.1-0.5 .mu.g of the substance increased the concn. of blood lymphocytes, hemagglutinins, hemolysins and antibody-forming cells.

L48 ANSWER 15 OF 20 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3  
1976:176167 Document No. 84:176167 A simple ultrasensitive method for the assay of cyclic AMP and cyclic GMP in tissues. Frandsen, E. K.; Krishna, G. (Nat'l. Heart Lung Inst., Nat'l. Inst. Health, Bethesda, Md., USA).

Life Sci., 18(5), 529-41 (English) 1976. CODEN: LIFSAK.  
AB A simple and rapid method for a highly sensitive radioimmunoassay of cyclic AMP (cAMP) and cGMP is described. The method is based on the observation that the affinity of the cyclic nucleotide antibodies for the 2'-O-succinyl or acetyl derivs. is considerably greater than that for the nonacylated cyclic nucleotides. With the present method, 3-10 femtmoles of cAMP and cyclic GMP can be assayed using commercially available antisera against cAMP or cGMP. A reproducible conversion of the cyclic nucleotides in aq. samples to the 2'-O-acylated deriv. is brought about by a simple 1-step addn. of premixed reagents contg. either succinic anhydride or Ac<sub>2</sub>O and triethylamine. The time required for succinylation or acetylation of 100 samples is 1 to < 5 min. Tissue exts. after purifn. through anion exchange resins do not interfere with the acylation. After acylation, labeled antigen and antiserum are added and incubated at 4.degree. for 15 hr. The bound antigen is sepd. from the unbound antigen by a simple EtOH pptn. using bovine serum albumin to ensure complete pptn. The specificity of the method was validated by the following criteria: treatment of the samples with purified phosphodiesterase results in complete loss of cyclic nucleotide immunoreactivity, cyclic nucleotide content was a linear function of the tissue wt., and in one instance using bovine rod outer segments, identical values of cGMP were obtained with the present method and a purely physical method (high pressure liq. chromatog.).

L48 ANSWER 16 OF 20 MEDLINE DUPLICATE 4  
75183995 Document Number: 75183995. The primary sequence of chicken myoglobin (*Gallus gallus*). Deconinck M; Peiffer S; Depreter J; Paul C; Schnek A G; Leonis J. BIOCHIMICA ET BIOPHYSICA ACTA, (1975 Apr 29) 386 (2)

567-75. Journal code: AOW. ISSN: 0006-3002. Pub. country: Netherlands.  
Language: English.

AB After enzymatic digestion of chicken myoglobin by trypsin, chymotrypsin or thermolysin, the separation of peptides was performed by column chromatography on various ion exchange resins. Each peptide was purified by high-voltage paper electrophoresis or by chromatography either on paper or on ion-exchange resin, and its complete amino acid sequence was then determined by the combined dansyl-Edman procedure and by endopeptidase digestions. The whole globin was submitted to automatic Edman degradation using the Beckman sequencer. Residues have been positioned from overlaps of sequence data between tryptic (T), chymotryptic (C) and thermolysin (Th) peptides. The stepwise degradation of the whole globin confirmed the alignment of the N-terminal third of the molecule. The combination of these different approaches has led to the complete determination of the 153 residues sequence forming the

polypeptide chain of chicken myoglobin. Comparison of the established chicken myoglobin structure with those from other species shows a conservation of structure, although the avian protein exhibits more variations in its amino acid sequence than has been found between other known myoglobins which all belong to mammalian species.

L48 ANSWER 17 OF 20 MEDLINE

75170370 Document Number: 75170370. Separation of dehydrogenases on polyaminomethylstyrene. Schopp W; Meinert S; Thyfronitou J; Aurich H. JOURNAL OF CHROMATOGRAPHY, (1975 Jan 29) 104 (1) 99-104. Journal code: HQF. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB The binding of dehydrogenases, especially alcohol dehydrogenase, and other

proteins to several ion exchangers and hydrophobic polymers was investigated. Quantitative parameters for the stability of the polymer-protein complexes (obtained from double reciprocal plots) indicate

a high but different affinity of many proteins for polyaminomethylstyrene.

The chromatography of a mixture of five dehydrogenases and human serum albumin on polyaminomethylstyrene is described.

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1975:404088 Document No. 83:4088 Column chromatography of hemoglobin peptides. Jones, Richard T. (Med. Sch., Univ. Oregon, Portland, Oreg., USA). CRC Crit. Rev. Clin. Lab. Sci., 5(1), 101-6 (English) 1974. CODEN: CRCLBH.

AB Tryptic peptides of Hb polypeptide chains can be isolated and purified by automatic peptide chromatog. The most satisfactory results were obtained by using aminoethylated chains and an initial peptide sepn. on either Bio-Rad Aminex A-5 or Spinco PA-35 resins by using a linear gradient of pyridine-HOAc developers. The peptides could be detected continuously in the column effluent with an automatic ninhydrin reaction system similar to that used for automatic amino acid anal. Partial alkaline hydrolysis could be effected before the ninhydrin reaction in order to detect peptides that were otherwise unreactive. Zones from the preparative chromatogram that contained >1 tryptic peptide could be purified further by rechromatography

on a column of cation-exchange or anion-exchange resin. Volatile org. buffers were used in order to facilitate the isolation of the purified peptides from the effluent solns.

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1970:495485 Document No. 73:95485 Isolation and characterization of rapidly labeled high molecular RNA from freely suspended callus cells of parsley (*Petroselinum sativum*). Seitz, U.; Richter, Gerd (Inst. Biol., Univ. Tuebingen, Tuebingen, Ger.). *Planta*, 92, 309-26 (German) 1970. CODEN: PLANAB.

AB Cells of callus tissue from roots of parsley were collected 5-9 days after

transfer so that they were in the stage of rapid cell multiplication. Cultures were grown in darkness with continuous aeration on a medium that contained sucrose, amino acids, accessory substances, and minerals.

Cells

previously held 12 hr on a P-free medium were placed 10-30 min in a  $^{32}\text{PO}_4^{3-}$  medium for expts. in rapid labeling or also held 2 hr on a P-free medium prior to a 36-hr exposure to  $^{32}\text{PO}_4^{3-}$ . Total nucleic acids were extd. into phenol, then into buffer at pH 7.5-9, and pptd. with EtOH.

The

ppt. was dissolved in Tris-borate-EDTA buffer followed by concn. of 2 ml by dialysis against 70% dextran for gel electrophoresis or in phosphate buffer pH 6.7 contg. 0.1M NaCl for column chromatog. on methylated albumin on diatomaceous earth (MAK). The MAK columns were eluted with solns. of 0.1M to 1.5M NaCl in 0.05M phosphate buffer pH 6.7. The eluates were collected in 5-ml portions in each of which the absorbance at 260 m.mu. and the  $^{32}\text{P}$  by scintillation counting were measured. High mol. wt. RNAs (sedimentation indices >18) were isolated from the solns. of total RNA in phosphate buffer at pH 6.7 by the procedure of Brawermann, et al. (1962). Portions of th solns. thus obtained were treated with serum albumin as carrier substance and pptd. by making the soln. to 5% with HClO<sub>4</sub>. The ppt. was hydrolyzed in 0.5N KOH and the unhydrolyzed material was pptd. with 70% HClO<sub>4</sub>. Portions of the solns. of the DNA purified on MAK columns were treated with unlabeled yeast DNA and pptd. with EtOH at -18.degree.. The ppt. was hydrolyzed with DNase, then adjusted to pH 9 and incubated with phosphodiesterase. Solns. of the nucleotides from either hydrolysis were neutralized and chromatographed on Dowex 1 .times. 2 ion exchange resin Cl form and eluted with 0.0 to 0.1N HCl.

In each of these fractions the absorbance at 254 m.mu. was detd. and the  $^{32}\text{P}$  was detd. by counting with an end-window tube. For electrophoresis the RNA solns. were stained with bromphenol blue, underlayered with TBE buffer pH 8.3, and applied to small tubes of acrylamide gel contg. acrylamide, methylenebisacrylamide, concd. TBE buffer, and NH<sub>4</sub> pesulfate. For 5 min 2.5 mA and then for 20 min 5 mA was passed through each tube after which the contents were pushed out and fixed in 1M HOAc. The RNAs of high mol. wt. from Brawermann procedure in 0.05M phosphate buffer, pH 6.7 were centrifuged in a sucrose gradient. For the centrifugates the absorbance at 260 m.mu. and the  $^{32}\text{P}$  by scintillation were detd. Rapid labeling appeared to be predominantly of RNA of high mol. wt., being found in the eluate fractions from the MAK columns after the ribosomal RNA,s from which it differed in having more AMP than GMP. Sepn. of these 2 RNAs on the sucrose gradients and by polyacrylamide electrophoresis indicated a sedimentation coeff. of .apprx.32S.

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1968:93412 Document No. 68:93412 Automatic chromatographic analysis of the dinitrophenylene and dinitrophenyl derivatives of lysine and tyrosine. Kunz, Heinz W.; Bernard, Colette F.; Gill, Thomas J., III (Harvard Med. Sch., Boston, Mass., USA). *J. Chromatogr.*, 32(4), 786-9 (English) 1968. CODEN: JOCRAM.

AB A method, useful in the study of proteins or other polypeptides, is described for sepg. the 5 dinitrophenyl(DNP) and

dinitrophenylene(DPE) derivs., O-DNP-tyrosine, O,O'-DPE-bis-tyrosine, N. $\epsilon$ -DNP-lysine, N. $\epsilon$ -O-DPE-lysine-tyrosine, and N. $\epsilon$ -DPE-bis-lysine, on an **anion-exchange resin**. The automated analyses were performed with a Technicon Amino Acid Analyzer under standard operating conditions since all 5 derivs. were ninhydrin pos. The only satisfactory system for resolving the 5 derivs. was AG 1-X2, 400-mesh (44-77 .mu.) **anion-exchange resin**, activated with 0.25M citric acid (pH 2.0), washed free of acid with H<sub>2</sub>O, equilibrated with 0.005M citrate buffer (pH 7.0), and packed into a column (60 cm. .times. 0.6 cm.) by using the citrate buffer. A **linear** citrate buffer pH gradient system of pH 7.0, 6.0, 5.5, and 5.0, was used; the column temp. was 30.degree. for 1 hr. and 35.degree. thereafter. The various derivs. were dissolved in 0.5M NaOH and **chromatographed** immediately. The column effluent was passed through the amino acid analyzer, and 1 anal. (by detg. the absorbance at 570 m.m.u.) required 8.5 hrs. The 5 derivs. were eluted in the order in which they are listed above, with their approx. elution times being 2.5, 3, 5, 7, and 7.5 hrs., resp.

L49 0 FILE MEDLINE  
L50 0 FILE CAPLUS  
L51 0 FILE BIOSIS  
L52 0 FILE EMBASE  
L53 0 FILE WPIDS

TOTAL FOR ALL FILES

L54 0 (ALPHA L INTEGRIN OR LFA IALPHA OR BETA 2 INTEGRIN OR B1 OR BP35) AND L16

=> s ansaldi d?/au,in;s lester p?/au,in  
'IN' IS NOT A VALID FIELD CODE  
L55 0 FILE MEDLINE  
L56 0 FILE CAPLUS  
L57 0 FILE BIOSIS  
'IN' IS NOT A VALID FIELD CODE  
L58 0 FILE EMBASE  
L59 0 FILE WPIDS

TOTAL FOR ALL FILES

L60 0 ANSALDI D?/AU,IN

'IN' IS NOT A VALID FIELD CODE  
L61 58 FILE MEDLINE  
L62 24 FILE CAPLUS  
L63 92 FILE BIOSIS  
'IN' IS NOT A VALID FIELD CODE  
L64 39 FILE EMBASE  
L65 4 FILE WPIDS

TOTAL FOR ALL FILES

L66 217 LESTER P?/AU,IN

=> s 166 and 116  
L67 0 FILE MEDLINE  
L68 0 FILE CAPLUS  
L69 0 FILE BIOSIS  
L70 0 FILE EMBASE